

University of Groningen

A light-responsive liposomal agent for MRI contrast enhancement and monitoring of cargo delivery

Reeßing, F.; Stuart, M C A; Samplonius, D F; Dierckx, R A J O; Feringa, B L; Helfrich, W; Szymanski, W

Published in:
Chemical communications (Cambridge, England)

DOI:
[10.1039/c9cc05516a](https://doi.org/10.1039/c9cc05516a)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Final author's version (accepted by publisher, after peer review)

Publication date:
2019

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Reeßing, F., Stuart, M. C. A., Samplonius, D. F., Dierckx, R. A. J. O., Feringa, B. L., Helfrich, W., & Szymanski, W. (2019). A light-responsive liposomal agent for MRI contrast enhancement and monitoring of cargo delivery. *Chemical communications (Cambridge, England)*, 55(72), 10784-10787.
<https://doi.org/10.1039/c9cc05516a>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Supporting information

A light-responsive liposomal probe for MR imaging and theranostics

F. Reeßing, M.C.A. Stuart, D.F. Samplonius, R.A.J.O. Dierckx, B.L. Feringa, W. Helfrich, W. Szymanski*

Contents

1. General information	2
2. Synthetic procedures.....	3
3. Preparation of liposomes	8
4. Cryo-TEM and EDX analysis	8
5. NMRD measurements	9
6. Determination of relaxation rates at 4.7 T	10
7. Determination of free Gd ³⁺ concentration	11
8. Cargo release from liposomes	12
9. Dynamic Light Scattering.....	13
10. <i>In vitro</i> analysis of liposome cytotoxicity	13
11. NMR spectra of new compounds	15
12. References	19

1. General information

Synthetic procedures

Starting materials, reagents and solvents were purchased from Sigma–Aldrich, Acros, Fluka, Fischer, TCI and were used as received. Solvents for the reactions were of quality puriss., p.a.. Anhydrous solvents were purified by passage through solvent purification columns (MBraun SPS-800). For aqueous solutions, deionized water was used. Thin Layer Chromatography analyses were performed on commercial Kieselgel 60, F₂₅₄ silica gel plates with fluorescence-indicator UV254 (Merck, TLC silica gel 60 F₂₅₄). For detection of components, UV light at $\lambda = 254$ nm or $\lambda = 365$ nm was used. Alternatively, oxidative staining using aqueous basic potassium permanganate solution (KMnO₄) or aqueous acidic cerium phosphomolybdic acid solution (Seebach's stain) was used. Drying of solutions was performed with MgSO₄ and volatiles were removed with a rotary evaporator. Flash column chromatography was performed with Silicagel, pore size 60 Å, 40-63 μ m particle size.

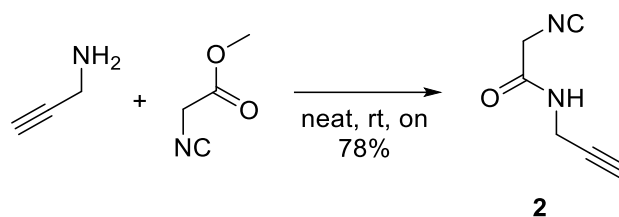
Analytical procedures

Nuclear Magnetic Resonance spectra were measured with an Agilent Technologies 400-MR (400/54 Premium Shielded) spectrometer (400 MHz). All spectra were measured at room temperature (22–24 °C). The multiplicities of the signals are denoted by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad signal). All ¹³C-NMR spectra are ¹H-broadband decoupled. High-resolution mass spectrometric measurements were performed using a Thermo scientific LTQ OrbitrapXL spectrometer with ESI ionization. The ions are given in m/z-units. Melting points were recorded using a Stuart analogue capillary melting point SMP11 apparatus. For spectroscopic measurements, solutions in Uvasol® grade solvents were measured in a 10 mm quartz cuvette. UV/Vis absorption spectra were recorded on an Agilent 8453 UV/Vis spectrophotometer with diode array detection. Temperature-control was exerted through a Peltier based temperature controlled cuvette holder (QuantumNorthwest). Fluorescence measurements were performed on a BioTek Synergy H1 microplate reader. ICP-OES analysis was performed on a Perkin Elmer Optima 7000 DV spectrometer. NMRD profiles were recorded on a Stelar 0.25 T FFC relaxometer SMARtracer.

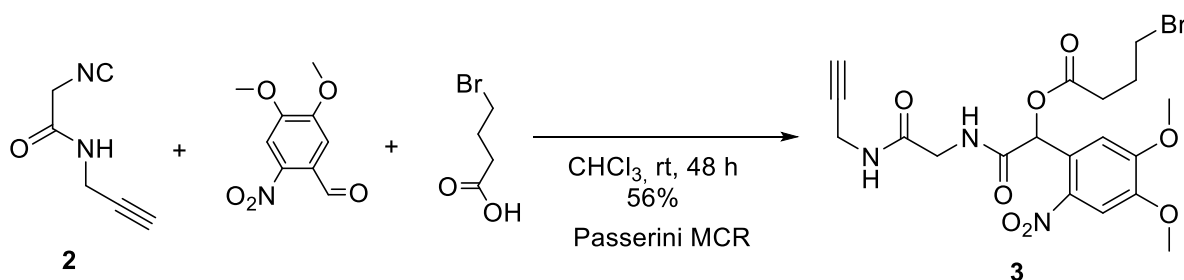
Irradiation sources

Irradiation experiments were performed with a $\lambda = 400$ nm LED system (3 x 330 mW, $\lambda_{\text{max}} = 401$ nm, FWHM 13.5 nm, Sahlmann Photochemical Solutions).

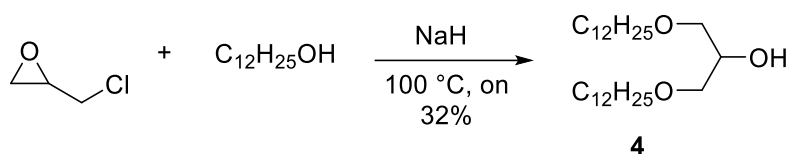
2. Synthetic procedures



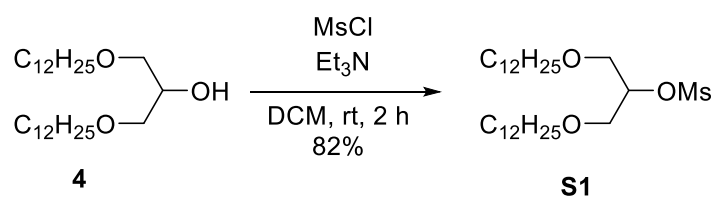
2: 2-Isocyano-*N*-(prop-2-yn-1-yl)acetamide. Prepared by a modification of a literature procedure.¹ A mixture of methyl isocyanoacetate (7.82 mmol, 711 μ L) and propargylamine (15.61 mmol, 1.00 mL) was stirred at room temperature overnight. The formed solid was filtered off and washed with diethyl ether to provide compound **2** as light brown powder (759 mg, 78%). R_f = 0.63 (pentane/AcOEt, 1:1, v/v); Mp. 109°C; ^1H NMR (400 MHz, DMSO- d_6): δ 3.15 (t, 4J = 2.4 Hz, 1H, CCH), 3.88 (dd, 3J = 5.6 Hz, 4J = 2.4 Hz, 2H, CH₂NH), 4.35 (s, 2H, CH₂NC), 8.61 (br s, 1H, NH). ^1H NMR spectrum is in agreement with published data.²



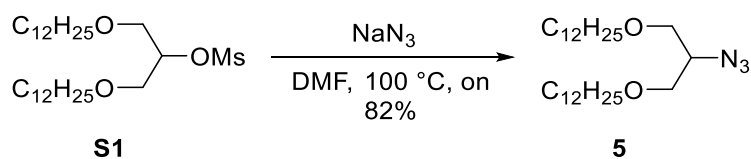
3: 1-(4,5-Dimethoxy-2-nitrophenyl)-2-oxo-2-((2-oxo-2-(prop-2-yn-1-ylamino)ethyl) amino)-ethyl 4-bromobutanoate. A solution of compound **2** (1.62 mmol, 200 mg), 6-nitroveratraldehyde (1.34 mmol, 285 mg) and 4-bromobutyric acid (1.62 mmol, 270 mg) in chloroform (2.7 mL) was stirred at room temperature for 48 h. The volatiles were evaporated and the product was purified by flash column chromatography (pentane/AcOEt, 8:2 to 2:8, v/v) to give a white powder (373 mg, 56%). R_f = 0.18 (pentane/AcOEt, 1:1, v/v); Mp. 142-143°C; ^1H NMR (400 MHz, DMSO- d_6): δ 2.03-2.12 (m, 2H, CH₂CH₂Br), 2.51-2.66 (m, 2H, CH₂CH₂CH₂Br), 3.09 (t, 4J = 2.4 Hz, 1H, CCH), 3.48-3.63 (m, 2H, CH₂Br), 3.74 (d, 3J = 6.0 Hz, 2H, CH₂NH), 3.82-3.85 (m, 2H, CH₂CC), 3.87 (s, 3H, CH₃O), 3.90 (s, 3H, CH₃O), 6.60 (s, 1H, CHO), 7.14 (s, 1H, ArH), 7.64 (s, 1H, ArH), 8.38 (t, 3J = 5.2 Hz, 1H, NH), 8.60 (t, 3J = 6.0 Hz, 1H, NH). ^{13}C NMR (100 MHz, DMSO- d_6): δ 28.1, 28.3, 32.3, 34.3, 42.1, 56.6, 56.8, 70.8, 73.6, 81.3, 108.7, 111.9, 124.7, 141.8, 149.0, 153.2, 167.7, 168.6, 171.5. HRMS (ESI+) calc. for $[\text{M}+\text{H}]^+$ (C₁₉H₂₃N₃O₈Br): 500.0663, found: 500.0652.



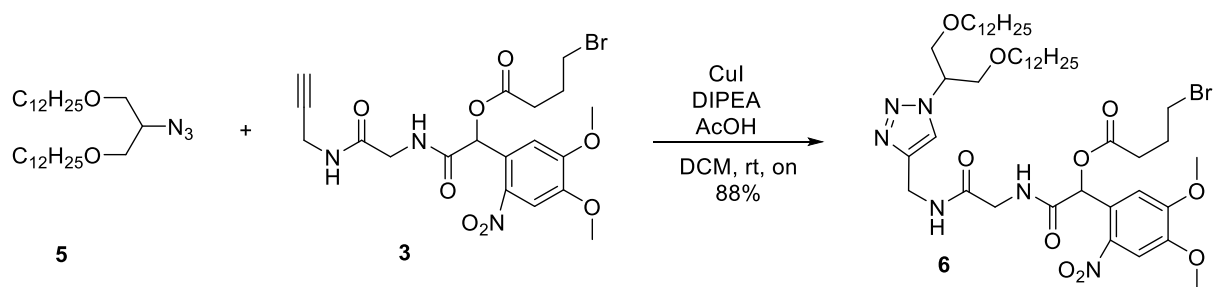
4: 1-(Dodecan-2-yloxy)-3-(dodecyloxy)propan-2-ol. NaH (60% in mineral oil, 8.58 mmol, 206 mg) was added to dodecan-1-ol (18.0 mmol, 3.35 g) at 100°C. After stirring for 30 min, epichlorohydrin (4.2 mmol, 0.39 g) was added dropwise and the mixture was stirred at 100°C overnight. H₂O was added to quench the reaction, followed by DCM (50 mL). The aqueous phase was extracted with DCM, the collected organic phases were washed with brine and dried over MgSO₄. Evaporation of volatiles and purification with flash column chromatography (pentane/AcOEt, 95:5, v/v) gave the product as a pale yellow solid (572 mg, 32%). *R_f* = 0.69 (pentane/AcOEt, 95:5, v/v); Mp. 30-32 °C; ¹H NMR (400 MHz, CDCl₃): δ 0.88 (t, 6H, CH₃), 1.26-1.29 (m, 36H, CH₃(CH₂)₉), 1.57 (m, 4H, CH₂CH₂O), 2.50 (d, 1H, OH), 3.41-3.51 (m, 8H, (CH₂OCH₂)₂CHOH), 3.92-3.96 (m, 1H, CHOH); ¹³C NMR (100 MHz, CDCl₃): δ 14.1, 22.7, 26.1, 29.3, 29.5, 29.6, 29.6, 29.7, 31.9, 69.5, 71.7, 71.8. ¹H NMR spectrum is in agreement with published data.³



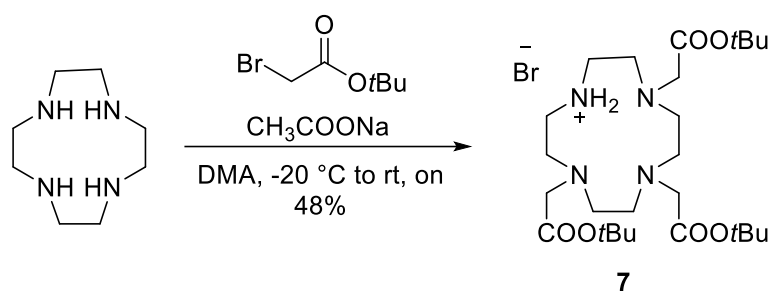
S1: 1-(Dodecan-2-yloxy)-3-(dodecyloxy)propan-2-yl methanesulfonate. A solution of methanesulfonyl chloride (1.46 mmol, 167 mg) in DCM (1 mL) was slowly added to a solution of compound **4** (1.17 mmol, 500 mg) and triethylamine (1.46 mmol, 148 mg) in DCM (3 mL) at room temperature. The mixture was stirred for 2 h. AcOEt was added and the organic phase was washed with H₂O and brine and dried (MgSO₄). Evaporation of volatiles gave the product as a pale yellow solid (484 mg, 82%). Mp. 57°C. (lit. 58-59 °C).⁴ ¹H NMR (400 MHz, CDCl₃): δ 0.88 (t, 6H, CH₂CH₃), 1.24-1.32 (m, 36H CH₃(CH₂)₉), 1.54-1.57 (m, 4H, CH₂CH₂O), 3.08 (s, 3H, SO₂CH₃), 3.41-3.51 (m, 4H, CH₂CHOHCH₂), 3.60-3.67 (m, 4H, CH₂CH₂O), 4.81 (q, 1H, CHOH). ¹H NMR spectrum is in agreement with published data.⁴



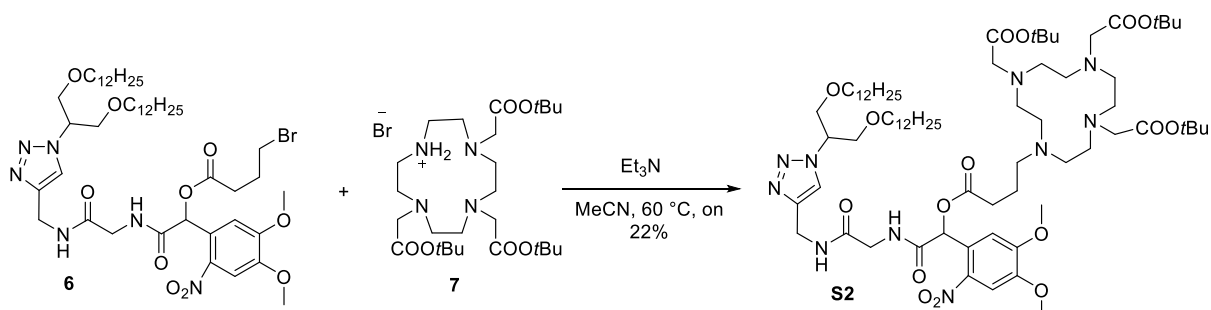
5: 1-(2-Azido-3-(dodecan-2-yloxy)propoxy)dodecane. Compound **S1** (0.79 mmol, 400 mg) and NaN_3 (6.30 mmol, 410 mg) were dissolved in DMF (3 mL) and the reaction mixture was stirred at 100°C overnight. AcOEt was added and the organic phase was washed with water and brine and dried (MgSO_4). Evaporation of the volatiles gave the product as a yellow oil (294 mg, 82%). $R_f = 0.53$ (AcOEt); ^1H NMR (400 MHz, CDCl_3): δ 0.88 (t, 6H, CH_3), 1.26-1.30 (m, 36H, $\text{CH}_3(\text{CH}_2)_9$), 1.54-1.60 (m, 4H, $\text{CH}_2\text{CH}_2\text{O}$), 3.43-3.57 (m, 8H, $\text{CH}_2\text{OCH}_2\text{CH}_2\text{AzideCH}_2\text{OCH}_2$), 3.65-3.71 (q, 1H, CH_2Azide). ^1H NMR spectrum is in agreement with published data.⁴



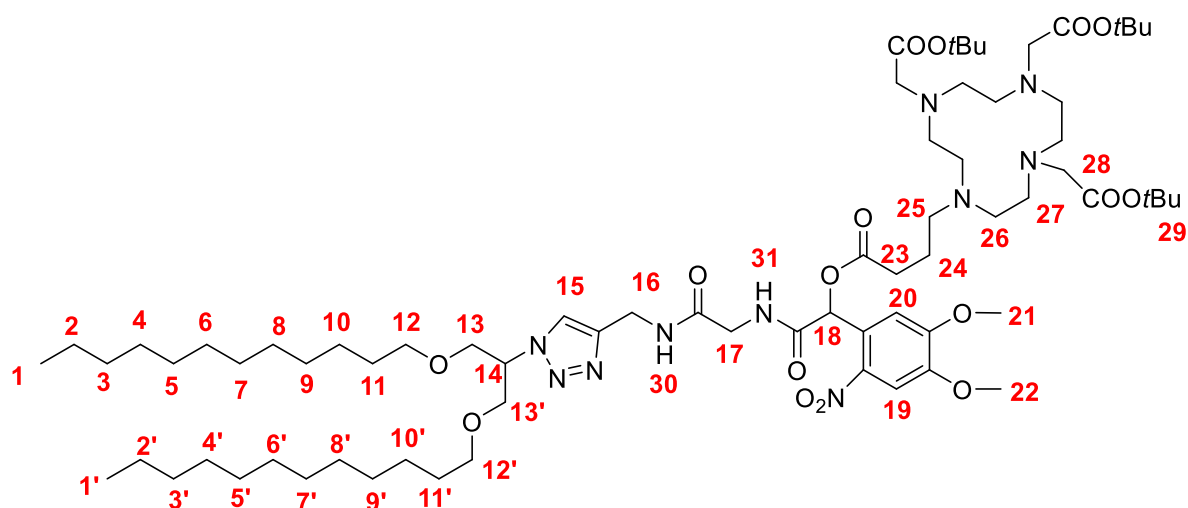
6: 2-((2-((1-(1,3-Bis(dodecyloxy)propan-2-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)-2-oxoethyl)amino)-1-(4,5-dimethoxy-2-nitrophenyl)-2-oxoethyl 4-bromobutanoate. The compound was prepared by a modification of a literature procedure for a different target.⁵ To a solution of compounds **3** (0.12 mmol, 61 mg), and **5** (0.15 mmol, 66 mg) were added of DIPEA (5.5 μmol , 0.72 mg) and acetic acid (5.5 μmol , 0.33 mg) in DCM (220 μL) and copper iodide (3 μmol , 0.6 mg). The reaction mixture was stirred at room temperature overnight. The product was purified by flash column chromatography (pentane/AcOEt 1:9 v/v to pure AcOEt) to obtain the product as a yellow oil (67.7 mg, 58%). $R_f = 0.80$ (AcOEt); ^1H NMR (400 MHz, CDCl_3): δ 0.88 (t, $^3J = 6.0$ Hz, 6H, $(\text{CH}_2)_{11}\text{CH}_3$), 1.26-1.28 (m, 40H, $\text{CH}_3(\text{CH}_2)_9$), 1.53 (m, 4H, $(\text{CH}_2)_9\text{CH}_2\text{CH}_2\text{O}$), 2.19 (q, $^3J = 7.0$ Hz 2H, $\text{CH}_2\text{CH}_2\text{Br}$), 2.60-2.73 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{Br}$), 3.39-3.54 (m, 5H, CH_2Br , $(\text{CH}_2)_{10}\text{CH}_2\text{O}$), 3.81 (d, $^3J = 8.0$ Hz, 4H, $\text{CH}_2\text{CH-TriazoleCH}_2$), 3.96 (s, 3H, OCH_3), 3.89 (dd, $^2J = 16.0$ Hz, $^3J = 6.0$ Hz 2H, HNCOCH_2NH), 4.01 (s, 3H, OCH_3), 4.06 (dd, $^2J = 16.0$ Hz, $^3J = 6.0$ Hz, 2H, HNCOCH_2NH), 4.51 (m, 2H, $\text{TriazoleCH}_2\text{NHCO}$), 4.85 (m, 1H, CH-Triazole), 6.74 (s, 1H, ArCHO), 7.13 (t, $^3J = 6.0$ Hz, 1H, NH), 7.20 (s, 1H, ArH), 7.38 (t, $^3J = 6.0$ Hz, 1H, NH), 7.60 (s, 1H, ArH), 7.70 (s, 1H, ArH); ^{13}C NMR (100 MHz, CDCl_3): δ 14.2, 22.8, 26.2, 27.7, 29.5, 29.6, 29.6, 29.7, 29.8, 29.8, 30.4, 32.0, 32.3, 32.6, 35.1, 43.1, 56.6, 56.8, 60.9, 69.3, 71.1, 71.8, 108.1, 111.1, 114.1, 122.5, 124.6, 124.9, 140.8, 149.2, 153.8, 167.7, 168.2, 171.7. HRMS (ESI+) calc. for $[\text{M}+\text{H}]^+$ ($\text{C}_{46}\text{H}_{77}\text{BrN}_6\text{O}_{10}$): 953.4957, found: 953.4966.



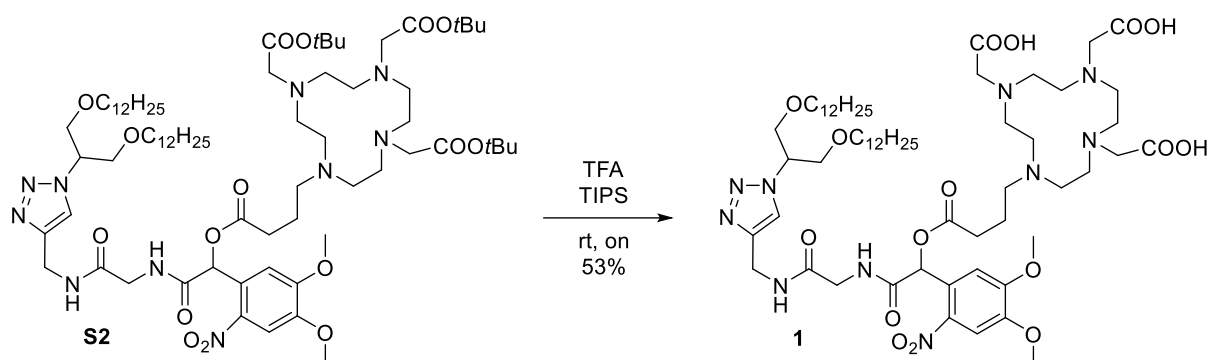
7: 4,7,10-Tris(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-ium bromide. The compound was prepared according to a literature procedure.⁶ A suspension of cyclen (5.81 mmol, 1.00 g) and sodium acetate (19.1 mmol, 1.57 g) in *N,N*-dimethylacetamide (DMA, 12 mL) was stirred at -20°C. A solution of *tert*-butyl bromoacetate (19.2 mmol, 2.83 mL) in DMA (4 mL) was added dropwise over 15 minutes at -20 °C. The reaction mixture was stirred at room temperature for 24 h, after which it was poured into water (60 mL), resulting in a clear solution. Solid KHCO₃ (30.0 mmol, 3.00 g) was added portion-wise and a precipitate was formed. The precipitate was collected by filtration and dissolved in DCM (5 mL) and the solution was washed with water (20 mL), dried over MgSO₄, filtered and concentrated to about 4-5 mL. Diethyl ether (50 mL) was added and compound **7** precipitated as a white solid (1.64 g, 48%). *R_f* = 0.63 (DCM/MeOH, 9:1, v/v). Mp. 179-181 °C. ¹H NMR (500 MHz, CDCl₃) δ 1.45 (s, 9H), 1.46 (s, 18H), 2.87 (m, 4H), 2.93 (m, 8H), 3.10 (m, 4H), 3.29 (s, 2H), 3.38 (s, 4H), 10.03 (br s, 1H). ¹H NMR spectrum is in agreement with published data.⁶



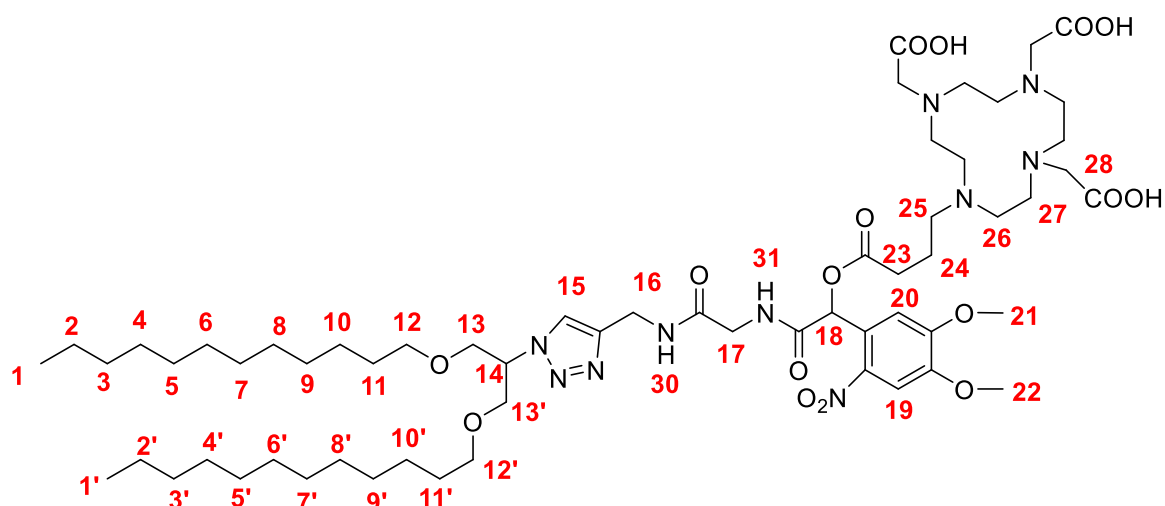
S2: Tri-*tert*-butyl 2,2',2''-(10-(4-(2-((1-(1,3-bis(dodecyloxy)propan-2-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)-2-oxoethyl)amino)-1-(4,5-dimethoxy-2-nitrophenyl)-2-oxoethoxy)-4-oxobutyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate. A solution of compound **6** (0.114 mmol, 109 mg), compounds **7** (0.1 mmol, 50 mg,) and triethylamine (0.24 mmol, 34 µL) in acetonitrile (3 mL) was stirred at 60°C overnight. The volatiles were evaporated and the product was purified by flash column chromatography (DCM/Methanol 98:2 – 90:10, v/v) to give a yellow oil (35 mg, 22%). *R_f* = 0.24 (DCM/MeOH, 95:5, v/v).



^1H NMR (400 MHz, CDCl_3): δ 0.88 (t, 6H, **1**, **1'**), 1.25 (m, 36H, **2-10**, **2'-10'**), 1.44-1.47 (m, 27H, **29**), 1.50-1.53 (m, 4H, **11**, **11'**), 1.83-3.18 (m, 28H, **23-28**), 3.41 (m, 4H, **12**, **12'**), 3.80-3.83 (m, 4H, **13**, **13'**), 3.93 (s, 3H, **21/22**), 3.99 (s, 3H, **21/22**), 4.05-4.10 (m, 2H, **17**), 4.52-4.53 (m, 2H, **16**), 4.83-4.84 (m, 1H, **14**), 6.82 (s, 1H, **18**), 7.29 (s, 1H, **19/20**), 7.51 (m, 1H, **30/31**), 7.56 (s, 1H, **15**), 7.81 (s, 1H, **19/20**), 8.46 (m, 1H, **30/31**); ^{13}C NMR (100 MHz, CDCl_3): δ 14.1, 22.7, 26.0, 27.9, 28.0, 28.0, 28.2, 29.4, 29.4, 29.5, 29.5, 29.6, 29.7, 31.9, 35.3, 43.2, 53.2, 53.4, 55.7, 56.4, 57.1, 60.6, 69.4, 70.6, 71.3, 71.7, 82.6, 83.0, 83.0, 108.0, 111.6, 122.5, 125.1, 141.2, 144.5, 148.9, 153.5, 168.1, 169.0, 172.3, 172.3. HRMS (ESI⁺) calc. for $[\text{M}+\text{H}]^+$ ($\text{C}_{72}\text{H}_{126}\text{N}_{10}\text{O}_{16}$): 1387.9426, found: 1387.9451.



1: 2,2',2''-(10-(4-(2-((2-(((1-(1,3-bis(dodecyloxy)propan-2-yl)-1H-1,2,3-triazol-4-yl) methyl)amino)-2-oxoethyl)amino)-1-(4,5-dimethoxy-2-nitrophenyl)-2-oxoethoxy)-4-oxobutyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid. A solution of compound **S2** (0.020 mmol, 25 mg) in trifluoroacetic acid (1.4 mL) and tri-*iso*-propylsilane (1.4 mL) was stirred at room temperature for 5 h. The volatiles were evaporated *in vacuo* and the residue was triturated with diethyl ether to give yellow solid (TFA salt, 16 mg, 53%). R_f = 0.73 (DCM/MeOH, 9:1, v/v).



^1H NMR (400 MHz, MeOD): δ 0.9 (t, 6H, **1**, **1'**), 1.28 (m, 42 H, **2-11**, **2'-11'**, **23**), 1.5 (m, 4 H, **24-25**), 3.05-3.7 (m, 22H, **26-28**), 3.21 (m, 4H, **12**, **12'**), 3.82 (m, 4H, **13**, **13'**), 3.88-3.99 (m, 2H, **17**), 3.93 (s, 3H, **22/21**), 3.96 (s, 3H, **22/21**), 4.48 (s, 2H, **16**), 4.9 (m, 1H, **14**), 6.81 (s, 1H, **18**), 7.25 (s, 1H, **19/20**), 7.69 (s, 1H, **19/20**), 7.91 (s, 1H, **15**). ^{13}C NMR (100 MHz, MeOD): δ 14.5, 23.8, 27.2, 30.5 (m), 30.8 (m), 31.6, 33.1, 35.7, 43.6, 57.0, 57.3, 62.5, 70.6, 72.5, 109.5, 113.1, 116.8 (TFA), 119.7 (TFA), 124.0, 125.5, 142.9, 145.7, 150.8, 154.9, 162.8 (TFA), 163.2 (TFA), 170.5, 171.1, 173.0. HRMS (ESI+) calc. for $[\text{M}+\text{H}]^+$ ($\text{C}_{60}\text{H}_{102}\text{N}_{10}\text{O}_{16}$): 1219.7556, found: 1219.7548.

3. Preparation of liposomes

The liposomes were prepared by mixing equimolar amounts of compound **1** with DOPC in chloroform. After evaporation of the organic solvent, TBS buffer (0.9 mL, 50 mM Tris, 150 mM NaCl, pH 7.5) was added to the dry lipid film to yield a concentration of 2.78 mM for both components. Five cycles of freezing, thawing and ultrasound sonication afforded the final liposomes. A solution of gadolinium trichloride in TBS buffer (2.5 mM GdCl_3 , 0.1 mL) was added to the liposomes and incubated for 2 hours during gentle stirring. Afterwards, the liposomes were purified from free gadolinium salts via dialysis (benzoylated dialysis tubing, MWCO: 2000, Sigma Aldrich).

4. Cryo-TEM and EDX analysis

Cryo-TEM was performed on a Tecnai T20 electron microscope (FEI) operated at 200 keV using a Gatan model 626 cryo-stage. Samples were vitrified with a vitrobot (FEI) on quantifoil 3.5/1 grids. Images were recorded on a slow-scan CCD camera under low-dose conditions. EDX analysis was done on the same microscope using a X-max 80T SDD detector (Oxford instruments). The ratio of

phosphorus to gadolinium signal was analyzed in two selected areas and determined to be 1: 1.76 (Gd/P).

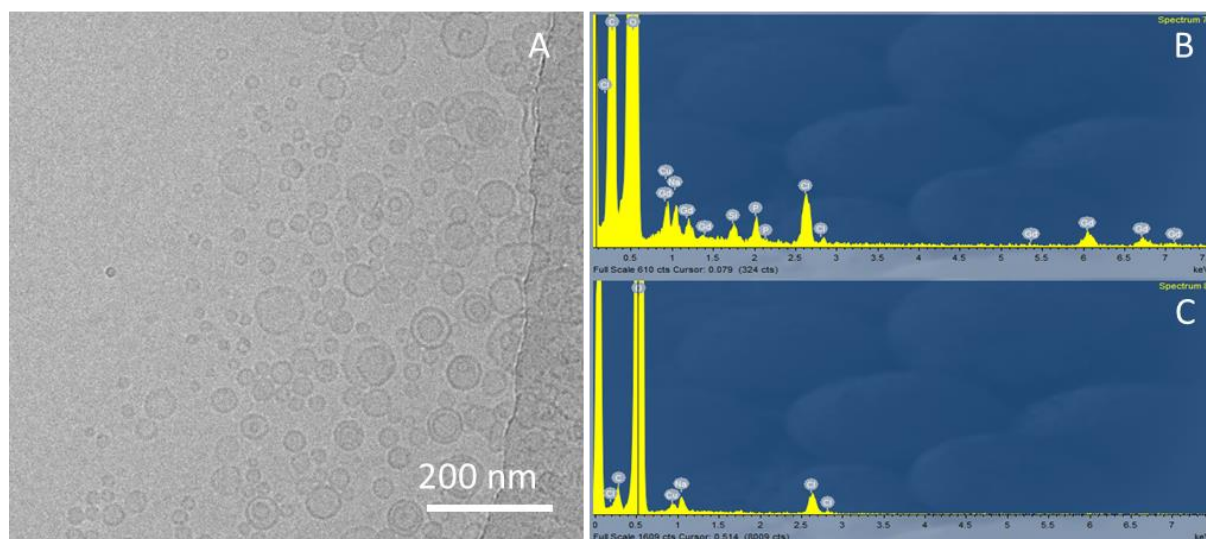


Fig. S1 (A) cryoTEM image of dialyzed liposomes, containing 50% DOPC and 50% compound **1**, with 1 equivalent of GdCl_3 added. (B) EDX spectrum of an area of liposome aggregation after addition of 2 equivalents of GdCl_3 and after dialysis. (C) EDX spectrum of the background of the same sample as in B. The absence of gadolinium signal in C indicates the selective accumulation in the liposomes through the successful Gd(III) -complex formation.

5. NMRD measurements

The liposomes were prepared as described above. The relaxation rates were determined over a frequency range of 0.01 – 10 MHz at 37 °C with 20 data points collected. The samples were irradiated in the NMRD vessel with $\lambda = 400$ nm for 60 min and NMRD profiles were recorded at the indicated time points. For the stability test, the sample was stored for up to four weeks in the dark at +4 °C.

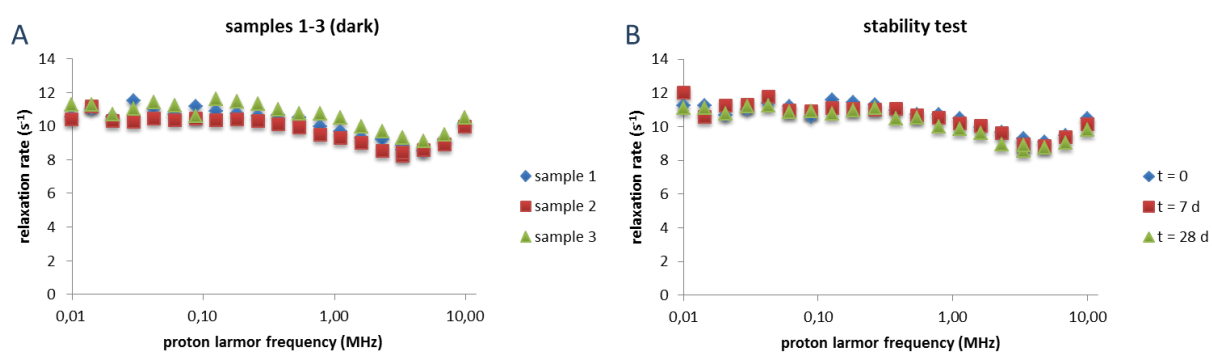


Fig. S2 (A) NMRD profiles of three independently prepared samples of liposomes containing Gd-1 (sample 1-3). (B) NMRD profiles of liposomes containing Gd-1 after preparation, after storage for one and four weeks. Spectrophotometric analysis

The absorbance was analyzed on a UV/Vis spectrophotometer. The liposomal formulation was irradiated as described above and the samples diluted with DMSO for analysis of the UV-Vis absorbance.

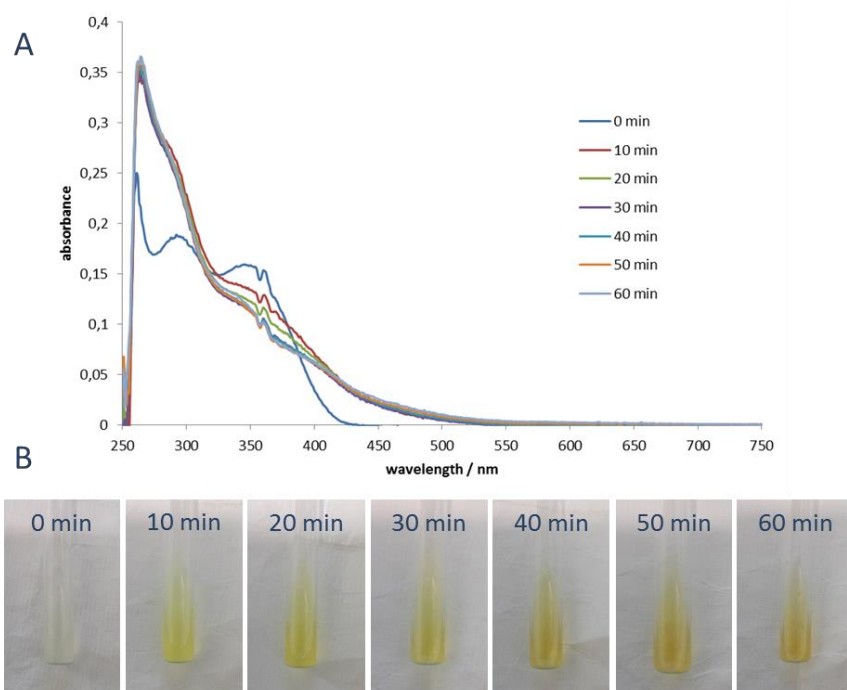


Fig. S3 (A) UV-Vis absorption spectra of liposomes containing Gd-1 before irradiation and after irradiation with $\lambda = 400$ nm for the indicated time points. (B) Visible change of the sample color upon irradiation

6. Determination of relaxation rates at 4.7 T

The relaxation rate of the liposomal formulation (prepared as described above) was determined on a Varian MercuryPlus spectrometer (4.7 T) at 37 °C using an inverse recovery experiment. The sample was irradiated in the NMR tube with $\lambda = 400$ nm light for 60 min and the relaxation rates were determined at the indicated time points.

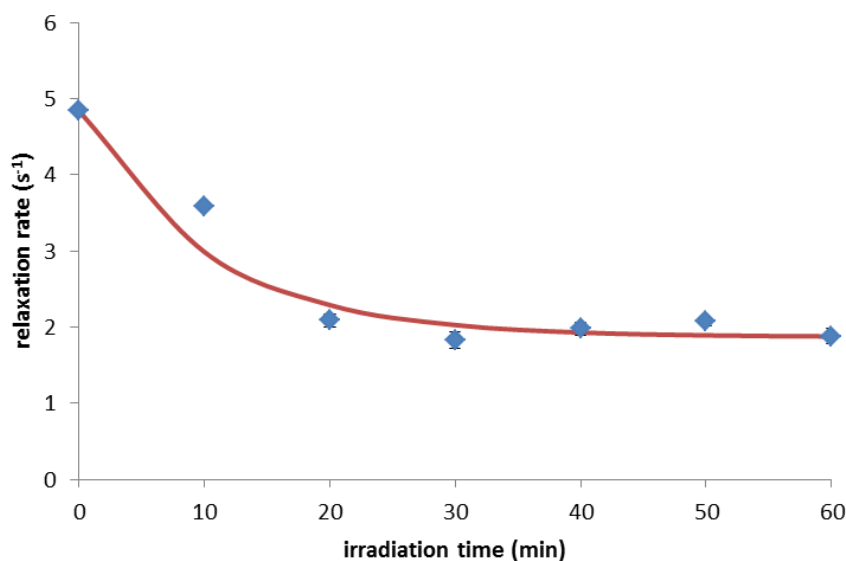


Fig. S4 Relaxation rate at 4.7 T in the dark and after irradiation for the indicated times

7. Determination of free Gd³⁺ concentration

The concentration of free Gd³⁺ was quantified by determination of the ratio of absorbance intensity at $\lambda = 573$ nm and $\lambda = 433$ nm of a Gd³⁺-xylenol orange complex⁷ in ammonium acetate buffer (100 mM, pH 5.8, 0.60 mM Xylenol Orange) using a microplate reader. The liposome sample was prepared as described above and diluted 1:20 with ammonium acetate buffer before analysis. The concentration of free Gd³⁺ before irradiation was determined as 0.029 μ M. After irradiation for 60 min with $\lambda = 400$ nm the free Gd³⁺ concentration was determined as 0.033 μ M.

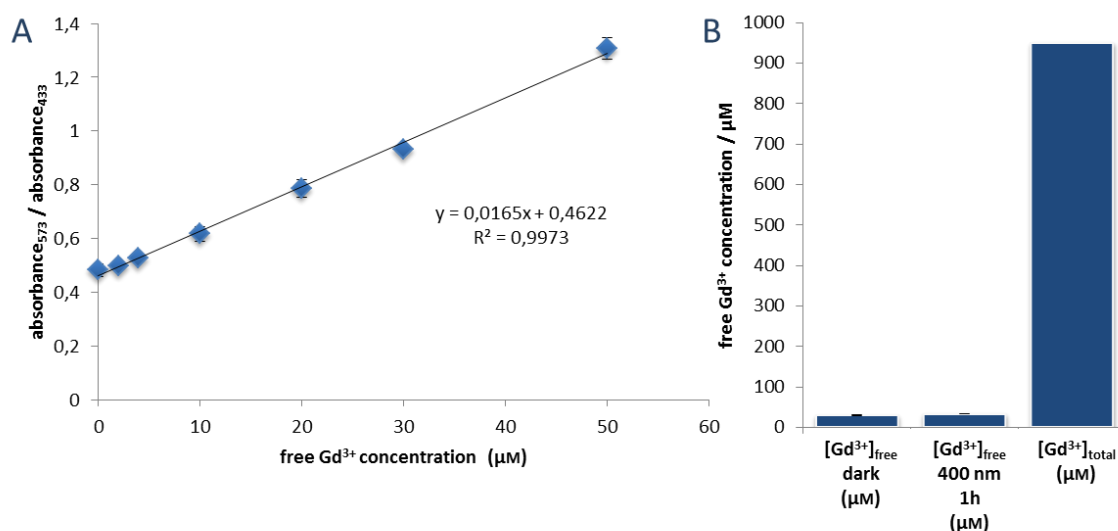


Fig. S5 (A) Calibration curve showing the ratio of absorbance intensity at $\lambda = 573$ nm and $\lambda = 433$ nm for increasing Gd³⁺ concentration in the presence of xylenol orange (0.60 mM). (B) Concentration of

free Gd^{3+} before and after irradiation with $\lambda = 400$ nm for 1 h and total gadolinium concentration (determined by ICP OES).

8. Cargo release from liposomes

The liposomes were prepared by adaptation of the method described above. A solution of calcein (0.1 M calcein, 0.1 M Tris buffer, pH 7.5) was used for hydration of the dry lipid film. The concentration of DOPC and compound **1** was 7 mM. The liposomes were purified from free calcein by size exclusion chromatography on a HiTrap desalting Sephadex G25 column using TBS buffer (0.1 M Tris, 0.15 M NaCl, pH 7.5) as elution buffer. The fluorescence ($\lambda_{\text{exc}} = 490$ nm, $\lambda_{\text{em}} = 520$ nm), indicating the amount of free calcein was analyzed on a microplate reader. At first the sample was kept in the dark for 60 minutes in order to assess the light-independent leakage of calcein. Subsequently, the sample was irradiated for 70 minutes with $\lambda = 400$ nm and the increase in fluorescence was checked every 10 minutes. At the end of the experiment, Triton X 100 (1% v/v) was added to determine the maximum calcein concentration. Liposomes consisting of only DOPC were prepared in the same way and analyzed as a control. Due to photobleaching of the fluorescence of the released calcein, it was not possible to determine the exact fraction of cargo release.

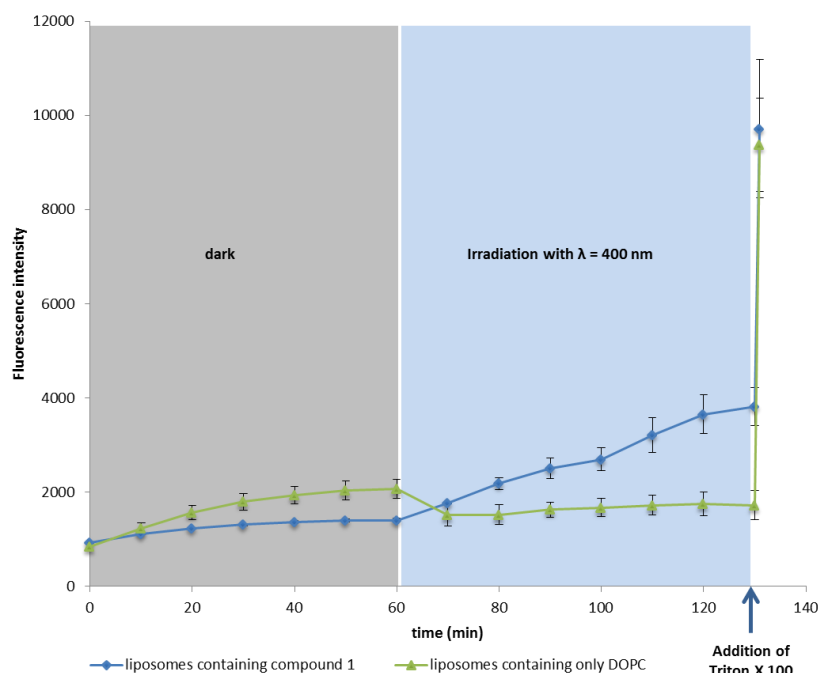


Fig. S6 Fluorescence intensity ($\lambda_{\text{exc}} = 490$ nm, $\lambda_{\text{em}} = 520$ nm) of calcein-loaded liposomes containing compound **1** in the dark, upon irradiation with $\lambda = 400$ nm and after addition of Triton X 100.

9. Dynamic Light Scattering

The size distribution of the liposomes was analyzed by Dynamic Light Scattering (DLS) using the Dynapro Nanostar apparatus, and the results were analyzed with Dynamics software, version 7. Before the analysis, the samples were diluted and centrifuged for 1 min at 10000 g to remove potential aggregates. As the irradiation progresses, the average size of the liposomes shifts towards smaller objects with a broader size distribution.

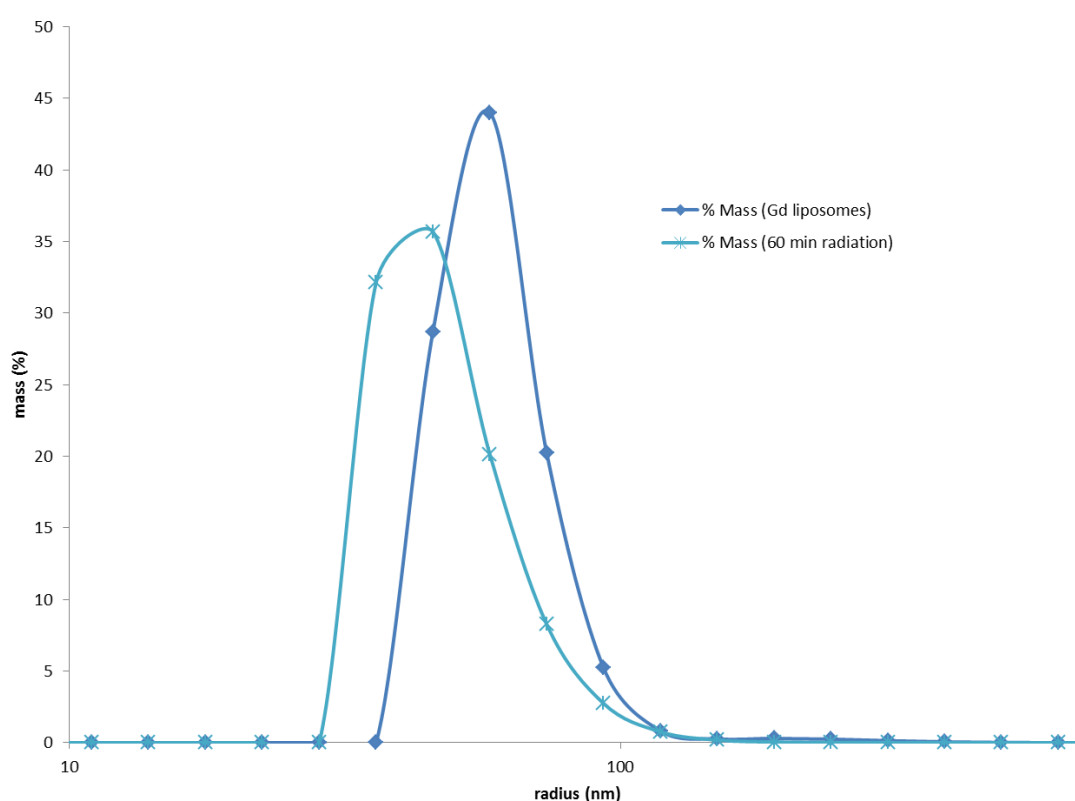


Fig. S7 Size of liposomes containing Gd-1 in the dark and after irradiation for 60 min.

10. In vitro analysis of liposome cytotoxicity

Primary colonic epithelium cells were obtained from Tebu-Bio (The Netherlands, 2–96115). Cells were cultured in RPMI-1640 (Lonza, Swiss), supplemented with 10% fetal calf serum (FCS, Thermo Scientific Waltham, MA)) at 37°C in a humidified 5% CO₂ atmosphere.

HUVEC cells were obtained from the UMCG Endothelial Cell Facility and were isolated and cultured by the method previously described.⁸

Monocytes: PBMCs were subjected to magnetic-activated cell sorting (MACS) with anti-CD14-beads and MS columns (Miltenyi Biotec). Macrophages: monocytes (1×10^6 /mL) were treated with 50 ng/mL M-CSF for 6 d, yielding M0 macrophages. M0 macrophages were subsequently stimulated with 50 ng/mL LPS and 20 ng/mL IFN γ to generate M1 macrophages.⁹

To test liposome induced cytotoxicity HUVEC, normal epithelial cells or M1 macrophages were seeded in 48 wells plate at density of $2,0 \times 10^4$ cells per well. The next day cells were incubated for 48 hours (M1 macrophages 24 hours) with either medium or 10 μ L liposomes solution which was irradiated with $\lambda = 400$ nm for the indicated time points. Treatment with high dose (70 μ M) taxol (Merck) was added as a positive control for apoptosis measurement. After incubation cells were harvested and stained with Annexin V-FITC (Immunotools, Germany) and propidium iodide (Merck) according to manufacturer's protocol and analyzed by flow cytometry (Guava, Merck).

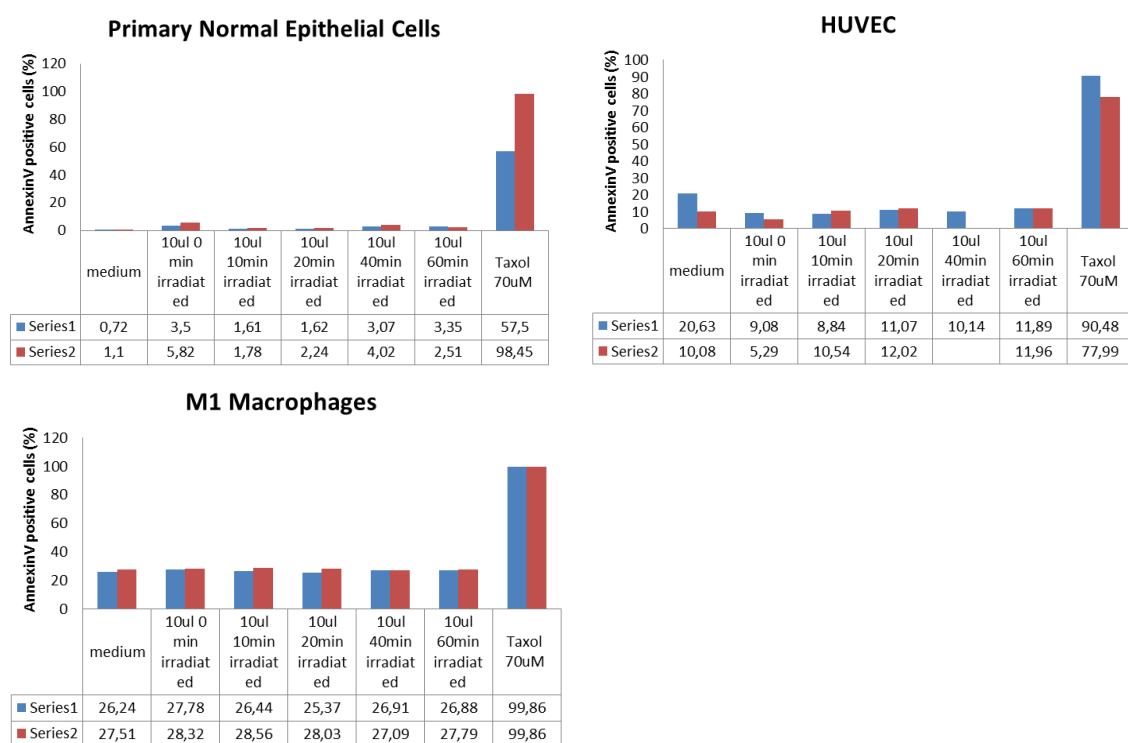
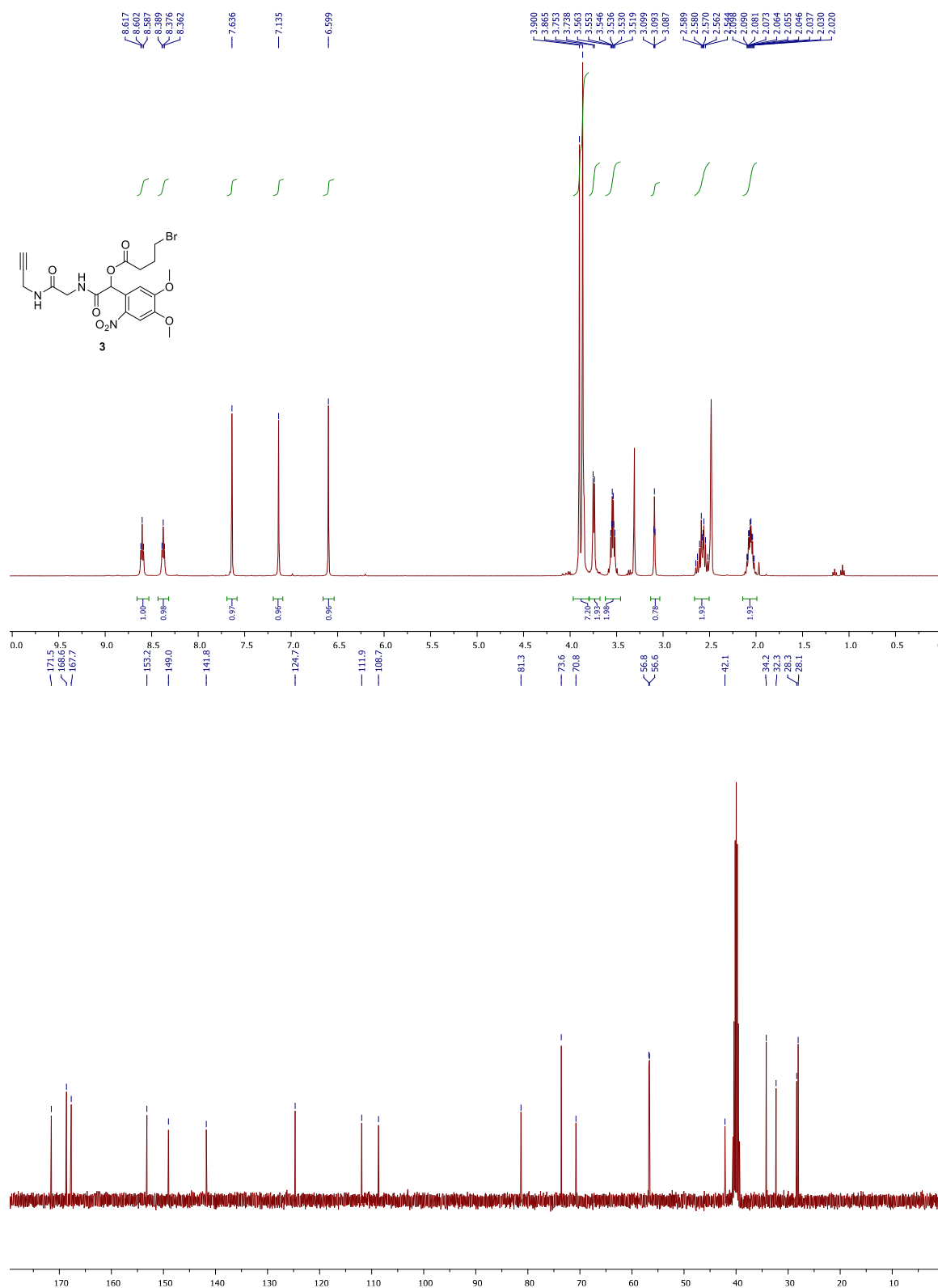


Fig. S8 Percentage of Annexin V/PI positive cells (analyzed by flow cytometry) after addition of medium or liposomes containing Gd-1 before and after irradiation with $\lambda = 400$ nm for the indicated times.

11. NMR spectra of new compounds

Compound **3**:



Chemical structure of compound 6:

CCCCCCCCCCCCOC(Cc1nn[nH]1)CCOC(=O)C2=CC(=C(C=C2)OC)C(=O)NCC(=O)NCC3=CC=CC=C3

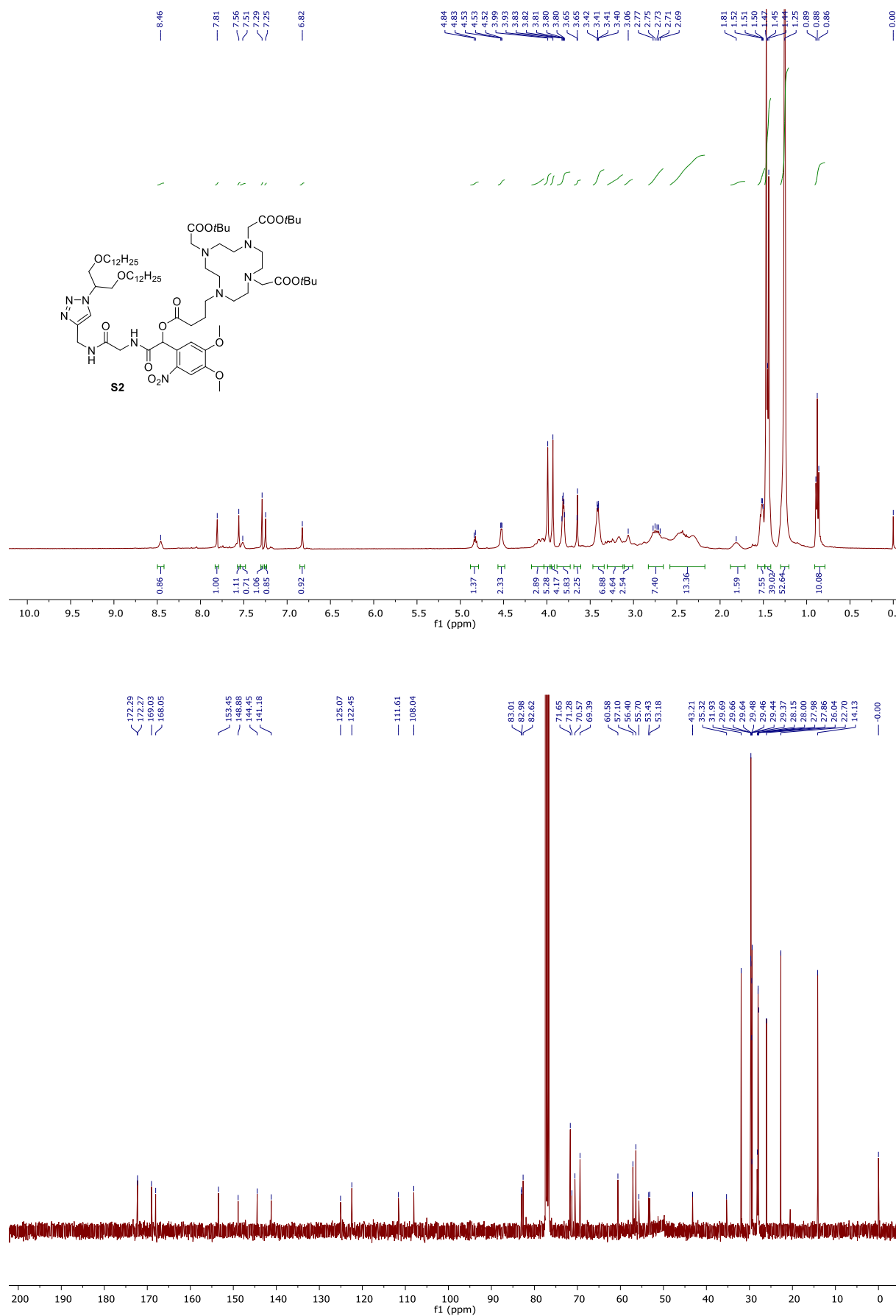
¹H NMR spectrum (CDCl₃):

Chemical Shift (ppm)	Integration
7.70	0.92
7.60	0.89
7.40	0.93
7.37	0.77
7.28	0.14
7.20	0.85
7.13	
6.99	
6.74	
4.85	0.89
4.51	1.78
4.01	1.37
3.96	2.62
3.86	2.62
3.80	3.39
3.52	5.32
3.51	
3.50	
3.48	
3.47	
3.45	
3.44	
3.43	
3.42	
3.41	
3.40	
3.39	
2.71	0.15
2.69	1.94
2.67	
2.66	
2.64	1.86
2.61	0.57
2.60	
2.23	3.69
2.21	1.62
2.19	38.26
2.18	
2.17	
1.55	6.00
1.53	
1.51	
1.43	
1.28	
1.27	
1.26	
1.25	
1.06	
0.88	
0.86	
-0.00	

¹³C NMR spectrum (CDCl₃):

Chemical Shift (ppm)
171.77
168.20
167.74
153.75
149.24
140.84
124.86
124.62
122.51
114.14
111.13
108.06
77.16
71.82
71.09
69.34
60.89
56.83
56.56
43.11
35.08
32.33
32.33
32.03
30.42
29.80
29.79
29.76
29.62
29.62
29.56
29.54
29.47
27.67
26.15
26.06
14.33

Compound S2:



Chemical Structure of 1: CCCCCCCCCCCCCCCCCOCc1nn(Cc2cc(C(=O)NCC(=O)Oc3cc(OC)cc(OC)c3)cc2)c1

¹H NMR Spectrum (Top): The spectrum shows peaks in the aromatic region (6.83-7.91 ppm) and aliphatic region (0.88-4.48 ppm). Integrations are provided for each peak.

Chemical Shift (ppm)	Integration
7.91	1.00
7.69	0.97
7.25	0.91
6.83	0.87
4.48	1.56
3.95	4.23
3.93	3.73
3.64	6.23
3.64	1.56
3.39	10.39
3.36	8.05
3.32	
3.31	
3.31	
3.30	
3.17	
2.67	1.64
2.59	
2.10	1.32
1.50	4.30
1.28	38.72
0.91	6.61
0.90	
0.88	

¹³C NMR Spectrum (Bottom): The spectrum shows peaks from 14.46 to 173.04 ppm.

Chemical Shift (ppm)
173.04
171.07
170.49
163.15
162.81
154.92
150.79
145.70
142.85
125.45
123.95
119.71
116.79
113.10
109.47
72.49
70.57
62.45
57.32
56.92
50.00
43.57
35.74
33.09
31.62
30.82
30.79
30.77
30.54
30.53
30.50
27.17
23.75
14.46

12. References

- 1 A. Dömling, B. Beck, T. Fuchs and A. Yazbak, *J. Comb. Chem.*, 2006, **8**, 872–80.
- 2 Y. Wang, Y. Wang and A. Dömling, 2016, **48**, 3701–3712.
- 3 H. Minamikawa, T. Murakami and M. Hato, *Chemistry and Physics of Synthesis of 1,3-di-O-alkyl-2-O-(3-glycosyl)glycerols bearing oligosaccharides as hydrophilic groups*, 1994.
- 4 H. Koyano, P. Bissel, K. Yoshihara, K. Ariga and T. Kunitake, *Chem. - A Eur. J.*, 1997, **3**, 1077–1082.
- 5 C. Shao, X. Wang, Q. Zhang, S. Luo, J. Zhao and Y. Hu, *J. Org. Chem.*, 2011, **76**, 6832–6836.
- 6 B. Jagadish, G. L. Brickert-Albrecht, G. S. Nichol, E. A. Mash and N. Raghunand, *Tetrahedron Lett.*, 2011, **52**, 2058–2061.
- 7 A. Barge, G. Cravotto, E. Gianolio and F. Fedeli, *Contrast Media Mol. Imaging*, 2006, **1**, 184–188.
- 8 A. Mulder, N. R. Blom, J. W. Smit, M. H. J. Ruijters, J. van der Meer, M. R. Halie and V. J. J. Bom, *Thromb. Res.*, 1995, **80**, 399–411.
- 9 D. Hendriks, Y. He, I. Koopmans, V. R. Wiersma, R. J. van Ginkel, D. F. Samplonius, W. Helfrich and E. Bremer, *Oncoimmunology*, 2016, **5**, e1202390.